Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

Antiretroviral Drug-Resistance Testing <mark>(Last updated November 1, 2012; last reviewed November 1, 2012)</mark>

Panel's Recommendations
• Antiretroviral (ARV) drug-resistance testing is recommended before initiation of therapy in all treatment-naive children **(AII)**. Genotypic resistance testing is preferred for this purpose **(AIII)**.
• ARV drug resistance testing is recommended before changing therapy because of treatment failure **(AI*)**.
• Resistance testing in patients with virological failure should be done while they are still on the failing regimen or within 4 weeks of discontinuation **(AII*)**.
• Phenotypic resistance testing should be used (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after virologic failure of successive ARV therapy regimens **(BIII)**.
• The absence of detectable resistance to a drug does not ensure that use of the drug will be successful. Consequently, previously used ARV agents and previous resistance test results should be reviewed when making decisions regarding the choice of new agents for patients with virologic failure **(AII)**.
• Viral coreceptor (tropism) assays should be used whenever the use of a CCR5 antagonist is being considered **(AI*)**. Tropism assays should also be considered for patients who demonstrate virologic failure while receiving therapy that contains a CCR5 antagonist **(AI*)**.
• Consultation with a specialist in pediatric HIV infection is recommended for interpretation of resistance assays when considering starting or changing an ARV regimen in pediatric patients **(AI*)**.
Rating of Recommendations: A = Strong; B = Moderate; C = Optional
Rating of Evidence: I = One or more randomized trials in children† with clinical outcomes and/or validated endpoints; I* = One or more randomized trials in adults with clinical outcomes and/or validated laboratory endpoints with accompanying data in children† from one or more well-designed, nonrandomized trials or observational cohort studies with long-term clinical outcomes; II = One or more well-designed, nonrandomized trials or observational cohort studies in children† with long-term outcomes; II* = One or more well-designed, nonrandomized trials or observational studies in adults with long-term clinical outcomes with accompanying data in children† from one or more similar nonrandomized trials or cohort studies with clinical outcome data; III = expert opinion
† Studies that include children or children and adolescents but not studies limited to postpubertal adolescents

HIV Drug-Resistance and Resistance Assays

HIV replication is a continuous process in most untreated patients, leading to the daily production of billions of viral particles. The goal of antiretroviral therapy (ART) is to suppress HIV replication as rapidly and fully as possible, indicated by a reduction in plasma HIV RNA to below the limit of detection of the most sensitive assays available. Unfortunately, mutations in HIV RNA readily arise during viral quantification because HIV reverse transcriptase (RT) is a highly error-prone enzyme. Consequently, ongoing replication in the presence of antiretroviral (ARV) drugs readily and progressively selects for strains of HIV with mutations that confer drug resistance.

Drug-resistance detection methods vary depending on the class of ARV agents. Viral coreceptor (tropism) assays have been successfully used to detect virus with tropism that will (CCR5 tropism) or will not (CXCR4 or mixed tropism) be blocked by CCR5 antagonists. Both genotypic assays and phenotypic assays <mark>currently</mark>

are used to detect the presence of virus that is resistant to inhibitors of the HIV RT, integrase (IN), or protease (PR). Clinical experience with testing for viral resistance to other agents is more limited, but genotypic assays that assess mutations in gp41 (envelope) genes also are commercially available. Experience is also limited with the use of commercially available genotypic and phenotypic assays in the evaluation of drug resistance in patients infected with non-B subtypes of HIV.[1]

Genotypic Assays

Genotypic assays for resistance to RT and PR inhibitors and integrase strand transfer inhibitors (INSTIs) are based on polymerase chain reaction (PCR) amplification and analysis of the RT, PR, and IN coding sequences present in HIV RNA extracted from plasma. Genotypic assays can detect resistance associated mutations in plasma samples containing approximately 1,000 copies/mL or more of HIV RNA and results generally are available within 1 to 2 weeks of sample collection.[2] Interpretation of test results requires knowledge of the mutations selected by different ARV drugs and of the potential for cross resistance to other drugs conferred by certain mutations. For some drugs, the genetic barrier to the development of resistance is low, and a single nucleotide mutation is enough to confer high-level resistance sufficient to remove any clinical utility of the drug. This is exemplified by resistance to nevirapine resulting from mutations in the HIV RT. Other mutations lead to drug resistance but simultaneously impair HIV replication. Clinically useful activity of the ARV agent may therefore remain, as demonstrated by evidence of continued clinical benefit from lamivudine in individuals with evidence of the high-level resistance engendered by the M184V RT mutation.[3] By contrast, HIV evolution to high-level resistance to some drugs is associated with the emergence of mutations that confer the resistance as well as compensatory mutations that allow the virus to replicate more efficiently in the presence of the ARV agent.

The International AIDS Society-USA (IAS-USA) and the Stanford University HIV Drug Resistance Database maintain lists of significant resistance-associated mutations relevant to currently available ARV drugs (see http://www.iasusa.org/resistance_mutations, or http://hivdb.stanford.edu). A variety of online tools that take into account the ability of some mutations selected by one drug to cause partial or full cross resistance with other drugs are now available to assist the provider in interpreting genotypic test results. Although the response to ART in children and adolescents is not always predicted by the results of genotypic resistance assays, clinical trials in adults have demonstrated the benefit of resistance testing combined with consultation with specialists in HIV drug resistance in improving virologic outcomes.[2, 4-10] Given the potential complexity of interpretation of genotypic resistance, it is recommended that clinicians consult with a specialist in pediatric HIV infection for assistance in the interpretation of genotypic results and design of an optimal new regimen.

Phenotypic Assays

Phenotypic resistance assays provide a more direct assessment of the impact on viral replication of mutations that are present among an individual's HIV variants. As they are most often performed, phenotypic assays involve PCR amplification of the RT, IN, PR, or gp41 envelope gene sequences from patient plasma and insertion of those amplified patient sequences into the backbone of a cloned strain of HIV that expresses a reporter gene. Replication of this recombinant virus in the presence of a range of drug concentrations is monitored by quantification of the reporter gene and is compared with replication of a reference drug susceptible HIV variant. The drug concentration that inhibits viral replication by 50% (that is, the mean inhibitory concentration, or IC_{50}) is calculated, and the ratio of the IC_{50} of test and reference viruses is reported as the fold increase in IC_{50} (i.e., fold resistance change). Automated, recombinant phenotypic assays that can produce results in 2 to 3 weeks are commercially available; however, they are more costly than genotypic assays.

Analytic techniques have also been developed to use the genotype to predict the likelihood of a drug-resistant phenotype. This bioinformatic approach, currently applicable for RT, IN, and PR inhibitor resistance only,

matches the pattern of mutations obtained from the patient sample with a large database of samples for which both genotype and phenotype are known. Therefore, the sample is assigned a predicted phenotype susceptibility (or "virtual phenotype") based on the data from specimens matching the patient's genotype. The primary limitations of this approach are that its predictive power depends upon the sensitivity of the genotypic methods used and the number of matches to the patient's genotype.

Tropism (Viral Coreceptor Usage) Assays

HIV enters cells by a complex, multistep process that involves sequential interactions between the HIV envelope protein molecules and the CD4 receptor, and then with either the CCR5 or CXCR4 coreceptor molecules, culminating in the fusion of the viral and cellular membranes. Viruses initially are CCR5 tropic in the majority of untreated individuals, including infants and children infected by mother-to-child transmission (MTCT) of HIV. However, a shift in coreceptor tropism often occurs over time, from CCR5 usage to either CXCR4 or both CCR5 and CXCR4 tropism (dual- or mixed-tropic; D/M-tropic). ARV-treated patients with extensive drug resistance are more likely to harbor detectable CXCR4- or D/M-tropic virus than untreated patients with comparable CD4 T lymphocyte (CD4 cell) counts.[11]

Resistance to CCR5 antagonists is detected using specialized phenotypic assay methods (Phenoscript [VIRalliance] and Trofile [Monogram Biosciences, Inc]). These assays involve the generation of recombinant viruses bearing patient-derived envelope proteins (gp120 and gp41). The relative capacity of these pseudoviruses to infect cells bearing the cell surface proteins CCR5 or CXCR4 is quantified based on the expression of a reporter gene.

Any indication of CXCR4 usage by virus detected in a patient is a contraindication to the use of the CCR5 antagonists as part of a therapeutic regimen. Coreceptor use assays should be performed before a CCR5 inhibitor is used and should be considered in patients exhibiting virologic failure on a CCR5 inhibitor such as maraviroc.

The Trofile assay takes about 2 weeks to perform and requires a plasma viral load ≥1,000 copies/mL. The initial version of the Trofile assay used during the clinical trials that led to the licensure of maraviroc was able to detect CXCR4-tropic virus with 100% sensitivity when present at a frequency of 10% of the plasma virus population but only 83% sensitivity when the variant was present at a frequency of 5%. In initial clinical trials of CCR5 antagonist drugs, this sensitivity threshold was not always sufficient to exclude the presence of clinically meaningful levels of CXCR4- or D/M-tropic virus in patients initiating a CCR5 inhibitor-based regimen. The current version of the Trofile assay has improved sensitivity and is able to detect CXCR4- or D/M-tropic virus representing as little as 0.3% of the plasma virus.[12, 13]

One of the tropism assays also can be performed following amplification of HIV sequences from peripheral blood DNA (Trofile-DNA [Monogram Biosciences, Inc.]) and may be useful when a change to a regimen containing a CCR5 antagonist is being considered for an individual with an undetectable plasma viral load.

Limitations of Current Resistance and Tropism Assays

Limitations of the genotypic, phenotypic, and phenotype-prediction assay approaches include lack of uniform quality assurance testing and high cost. In addition, drug-resistant variants are likely to exist at low levels in every HIV-infected patient. Drug-resistant viruses that constitute <10% to 20% of the circulating virus population or are present in the reservoir of latently infected cells may not be detected by any of the currently available commercial resistance assays.[14] Consequently, a review of the past use of ARV agents is important in making decisions regarding the choice of new agents for patients with virologic failure.

Genotypic assays have been proposed as an alternative approach to detemining the tropism of plasma HIV. However, they are not currently recommended because the limited experience with this approach indicates that the sensitivity and specificity are lower than phenotypic tropism assays.[15]

Although drug resistance may be detected in infants, children, and adults who are not receiving therapy at the time of the assay, loss of detectable resistance and reversion to predominantly wild-type virus often occur in the first 4 to 6 weeks after ARV drugs are stopped.[16-18] As a result, resistance testing is of greatest value when performed within 4 weeks after drugs are discontinued. The absence of detectable resistance to a drug at the time of testing does not ensure that future use of the drug will be successful,[19] especially if the agent shares cross resistance with drugs previously used. It may be prudent to repeat resistance testing if an incomplete virological response to a new treatment regimen is observed in an individual with prior treatment failure(s) (see Management of Treatment-Experienced Infants, Children, and Adolescents).

Use of Resistance Assays in Determining Initial Treatment

MTCT transmission and behavioral transmission of drug-resistant HIV strains have been well documented and are associated with suboptimal virologic response to initial ART.[20-24] Drug-resistant variants of HIV may persist for months after birth in infected infants[25] and impair the response to ART.[26] Consequently, ARV drug-resistance testing is recommended for all treatment-naive children before therapy is initiated. Genotypic testing is preferred in this setting because it may reveal the presence of both resistance mutations and polymorphisms that facilitate the replication of drug-resistant virus.

Use of Resistance Assays in the Event of Virologic Failure

Several studies in adults[2, 4-10] have indicated that early virologic responses to salvage regimens were improved when results of resistance testing were available to guide changes in therapy, compared with responses observed when changes in therapy were guided only by clinical judgment. Although not yet confirmed in children,[27] resistance testing appears to be a useful tool in selecting active drugs when changing ARV regimens in cases of virologic failure. Resistance testing also can help guide treatment decisions for patients with suboptimal viral load reduction because virologic failure in the setting of combination ART may be associated with resistance to only one component of the regimen.[1] Poor adherence should be suspected when no evidence of resistance to a failing regimen is identified (see Management of Treatment-Experienced Infants, Children, and Adolescents).

References

1. Hirsch MS, Gunthard HF, Schapiro JM, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. *Clin Infect Dis*. Jul 15 2008;47(2):266-285. Available at http://www.ncbi.nlm.nih.gov/pubmed/18549313.

2. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. *Lancet*. Jun 26 1999;353(9171):2195-2199. Available at http://www.ncbi.nlm.nih.gov/pubmed/10392984.

3. Campbell TB, Shulman NS, Johnson SC, et al. Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. *Clin Infect Dis*. Jul 15 2005;41(2):236-242. Available at http://www.ncbi.nlm.nih.gov/pubmed/15983922.

4. Baxter JD, Mayers DL, Wentworth DN, et al. A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS. *AIDS*. Jun 16 2000;14(9):F83-93. Available at http://www.ncbi.nlm.nih.gov/pubmed/10894268.

5. Cingolani A, Antinori A, Rizzo MG, et al. Usefulness of monitoring HIV drug resistance and adherence in individuals failing highly active antiretroviral therapy: a randomized study (ARGENTA). *AIDS*. Feb 15 2002;16(3):369-379. Available at http://www.ncbi.nlm.nih.gov/pubmed/11834948.

6. Cohen CJ, Hunt S, Sension M, et al. A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy. *AIDS*. Mar 8 2002;16(4):579-588. Available at http://www.ncbi.nlm.nih.gov/pubmed/11873001.

7.   Meynard JL, Vray M, Morand-Joubert L, et al. Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. *AIDS*. Mar 29 2002;16(5):727-736. Available at http://www.ncbi.nlm.nih.gov/pubmed/11964529.

8.   Vray M, Meynard JL, Dalban C, et al. Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1-infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS 088). *Antivir Ther*. Oct 2003;8(5):427-434. Available at http://www.ncbi.nlm.nih.gov/pubmed/14640390.

9.   Wegner SA, Wallace MR, Aronson NE, et al. Long-term efficacy of routine access to antiretroviral-resistance testing in HIV type 1-infected patients: results of the clinical efficacy of resistance testing trial. *Clin Infect Dis*. Mar 1 2004;38(5):723-730. Available at http://www.ncbi.nlm.nih.gov/pubmed/14986258.

10.   Tural C, Ruiz L, Holtzer C, et al. Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. *AIDS*. Jan 25 2002;16(2):209-218. Available at http://www.ncbi.nlm.nih.gov/pubmed/11807305.

11.   Hunt PW, Harrigan PR, Huang W, et al. Prevalence of CXCR4 tropism among antiretroviral-treated HIV-1-infected patients with detectable viremia. *J Infect Dis*. Oct 1 2006;194(7):926-930. Available at http://www.ncbi.nlm.nih.gov/pubmed/16960780.

12.   Su Z, Gulick RM, Krambrink A, et al. Response to vicriviroc in treatment-experienced subjects, as determined by an enhanced-sensitivity coreceptor tropism assay: reanalysis of AIDS clinical trials group A5211. *J Infect Dis*. Dec 1 2009;200(11):1724-1728. Available at http://www.ncbi.nlm.nih.gov/pubmed/19874179.

13.   Cooper DA, Heera J, Goodrich J, et al. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine, for the treatment of antiretroviral-naive subjects with CCR5-tropic HIV-1 infection. *J Infect Dis*. Mar 15 2010;201(6):803-813. Available at http://www.ncbi.nlm.nih.gov/pubmed/20151839.

14.   Gardner EM, Burman WJ, Steiner JF, Anderson PL, Bangsberg DR. Antiretroviral medication adherence and the development of class-specific antiretroviral resistance. *AIDS*. Jun 1 2009;23(9):1035-1046. Available at http://www.ncbi.nlm.nih.gov/pubmed/19381075.

15.   Prosperi MC, Bracciale L, Fabbiani M, et al. Comparative determination of HIV-1 co-receptor tropism by Enhanced Sensitivity Trofile, gp120 V3-loop RNA and DNA genotyping. *Retrovirology*. 2010;7:56. Available at http://www.ncbi.nlm.nih.gov/pubmed/20591141.

16.   Devereux HL, Youle M, Johnson MA, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. *AIDS*. Dec 24 1999;13(18):F123-127. Available at http://www.ncbi.nlm.nih.gov/pubmed/10630517.

17.   Miller V, Sabin C, Hertogs K, et al. Virological and immunological effects of treatment interruptions in HIV-1 infected patients with treatment failure. *AIDS*. Dec 22 2000;14(18):2857-2867. Available at http://www.ncbi.nlm.nih.gov/pubmed/11153667.

18.   Verhofstede C, Wanzeele FV, Van Der Gucht B, De Cabooter N, Plum J. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. *AIDS*. Dec 24 1999;13(18):2541-2546. Available at http://www.ncbi.nlm.nih.gov/pubmed/10630523.

19.   Benson CA, Vaida F, Havlir DV, et al. A randomized trial of treatment interruption before optimized antiretroviral therapy for persons with drug-resistant HIV: 48-week virologic results of ACTG A5086. *J Infect Dis*. Nov 1 2006;194(9):1309-1318. Available at http://www.ncbi.nlm.nih.gov/pubmed/17041858.

20.   Borroto-Esoda K, Waters JM, Bae AS, et al. Baseline genotype as a predictor of virological failure to emtricitabine or stavudine in combination with didanosine and efavirenz. *AIDS Res Hum Retroviruses*. Aug 2007;23(8):988-995. Available at http://www.ncbi.nlm.nih.gov/pubmed/17725415.

21.   Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. *N Engl J Med*. Jul 15 2004;351(3):229-240. Available at http://www.ncbi.nlm.nih.gov/pubmed/15247339.

22. Kuritzkes DR, Lalama CM, Ribaudo HJ, et al. Preexisting resistance to nonnucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment-naive HIV-1-infected subjects. *J Infect Dis*. Mar 15 2008;197(6):867-870. Available at http://www.ncbi.nlm.nih.gov/pubmed/18269317.

23. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med*. Aug 8 2002;347(6):385-394. Available at http://www.ncbi.nlm.nih.gov/pubmed/12167680.

24. Pozniak AL, Gallant JE, DeJesus E, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes--a 96-week analysis. *J Acquir Immune Defic Syndr*. Dec 15 2006;43(5):535-540. Available at http://www.ncbi.nlm.nih.gov/pubmed/17057609.

25. Persaud D, Palumbo P, Ziemniak C, et al. Early archiving and predominance of nonnucleoside reverse transcriptase inhibitor-resistant HIV-1 among recently infected infants born in the United States. *J Infect Dis*. May 15 2007;195(10):1402-1410. Available at http://www.ncbi.nlm.nih.gov/pubmed/17436219.

26. Palumbo P, Lindsey JC, Hughes MD, et al. Antiretroviral treatment for children with peripartum nevirapine exposure. *N Engl J Med*. Oct 14 2010;363(16):1510-1520. Available at http://www.ncbi.nlm.nih.gov/pubmed/20942667.

27. Green H, Gibb DM, Compagnucci A, et al. A randomized controlled trial of genotypic HIV drug resistance testing in HIV-1-infected children: the PERA (PENTA 8) trial. *Antivir Ther*. 2006;11(7):857-867. Available at http://www.ncbi.nlm.nih.gov/pubmed/17302248.